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Photocleavable Peptide-Oligonucleotide Conjugates for Protein Kinase Assays by MALDI-TOF MS†

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Abstract

Robust methods for highly parallel, quantitative analysis of cellular protein tyrosine kinase activities may provide tools critically needed to decipher oncogenic signaling, discover new targeted drugs, diagnose cancer and monitor patients. Here, we describe proof-of-principle for a novel protein kinase assay with potential to answer these challenges. MALDI-TOF mass spectrometry provides an ideal tool for label-free multiplexed analysis of peptide phosphorylation, but is poorly matched to homogeneous assays and complex samples. Thus, we conjugated a common oligonucleotide tag to multiple peptide substrates, offering efficient capture from solution-phase kinase reactions by annealing to the complementary sequence tethered to PEG-passivated superparamagnetic microparticles. To enable reversible conjugation, we developed a novel bifunctional cross-linker allowing simple and efficient preparation of photocleavable peptide-oligonucleotide conjugates. After washing away contaminants and photorelease, MALDI-TOF analysis yielded relative phosphorylation of each peptide with high sensitivity and specificity. Validating the hybridization-mediated multiplexed kinase assay, when three peptide substrate-oligonucleotide conjugates were mixed with the tyrosine kinase c-Abl and ATP, we readily observed their differential phosphorylation yet measured a common IC₅₀ for the Abl kinase inhibitor imatinib. This new assay enables analysis of protein kinase activities in a multiplexed format amenable to screening inhibitors against multiple kinases in parallel, an important capability for drug discovery and predictive diagnostics.

Introduction

It has long been appreciated that the uncontrolled growth of cancer cells is tightly linked to the activity of deregulated protein tyrosine kinases and the resulting altered patterns of protein tyrosine phosphorylation.^{1–2} As such, both receptor and non-receptor tyrosine kinases have emerged as key targets for cancer therapy.^{3–6} Despite their initial promise, most small-molecule inhibitors targeting oncogenic tyrosine kinases that have reached

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clinical trials in the past decade have yielded a common pattern of unpredictable responses, unanticipated side effects, and rapid development of resistance after initial response. Indeed, most cancer patients eventually relapse in the face of molecularly targeted treatments.⁷ Perhaps contributing to the pattern of recent failures is the continuing use of tools for drug discovery that provide inadequate models for disease and a corresponding lack of companion diagnostics to predict and monitor patient responses. To answer these gaps, new and powerful quantitative kinase assay techniques are needed for use in mechanistic discovery, drug screening and clinical diagnostics.

Protein kinase assays are typically categorized into homogeneous and heterogeneous formats based on their assay geometry.^{8–11} In homogeneous assays, both the reaction and detection are performed in solution phase. Such methods offer the advantages of fast reaction kinetics, potential for real-time detection and ready implementation in high throughput screening (HTS) assays. However, the common use of luminescent or fluorescent probes, typically based on antibody recognition,^{12–17} offers only indirect, label-dependent detection of tyrosine phosphorylation, thereby compromising dynamic range, and sensitivity and specificity and providing limited potential for multiplexing. The application of modern chemical approaches has led to several advances in detection methods for homogenous assays^{8, 12, 18–36} but only limited progress has been reported toward multiplexing of substrates and/or kinases.^{31–32, 37–39} Notably, Imperiali and colleagues³⁹ reported a fluorescence-based homogeneous assay enabling multiplexed kinase detection in cell lysates. Alternatively, heterogeneous formats, also called solid-phase assays, exploit substrates tethered to a matrix or onto a surface, enabling phosphorylation to be performed under one condition and detection under another one. A major concern is that phosphorylation kinetics may be affected by limited substrate accessibility. Further, the requirement for increased sample handling can be problematic for HTS. Nonetheless, the disadvantages of solid-phase assays are offset by enhanced sensitivity and specificity and improved quantitation.

Label-free detection of phosphorylation is likely a prerequisite for highly multiplexed tyrosine kinase assays. Mass spectrometry has been demonstrated to be a most promising label-free approach for the analysis of protein phosphorylation.^{40–42} Applying this principle to homogeneous assays, Kubota et al.³² developed a single-reaction strategy to measure the differential phosphorylation of 90 peptide substrates using electrospray ionization mass spectrometry. We^{24–25, 43–45} and others^{46–48} have exploited matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) as a tool for label-free detection of peptide phosphorylation in heterogeneous assays with substrates tethered to MALDI targets, self-assembled monolayers, microarrays, microparticles and other geometries. MALDI-TOF MS-based phosphorylation detection is comparatively simple, does not require elaborate chromatography-based separation steps, is compatible with HTS and has the potential for unlimited multiplexing. However, MALDI-TOF MS-based detection is poorly matched to homogenous kinase assays because multiple components of kinase assays including salts, buffers, and biomacromolecules can suppress ionization and detection of analyte peptide ions and skew apparent phosphorylation levels.

A clever hybrid strategy was reported by Zhao et al.,³¹ who demonstrated a highly multiplexed hybrid kinase assay involving solution-phase phosphorylation of peptide-oligonucleotide substrates followed by DNA-directed assembly onto an oligonucleotide microarray and detection by anti-phosphotyrosine antibody and fluorescence detection. Toward integrating the advantages of homogeneous assays and label-free MALDI-TOF detection, we have developed an assay based on solution-phase phosphorylation of peptide-oligonucleotide conjugates, followed by conjugate separation by annealing to oligonucleotide-coated magnetic beads, subsequent photorelease, and detection by MALDI-

TOF MS (Figure 1). Our strategy combines the advantages of both solid- and solution-phase geometries, wherein solution-phase phosphorylation is followed by solid-phase capture, and offers the potential for HTS analysis.

Results and discussion

Peptide-oligonucleotide conjugates can be prepared by diverse methods.^{31, 49–55} Though strictly feasible, total stepwise solid-phase synthesis is not straightforward due to incompatibilities between standard peptide and nucleotide synthesis chemistries. A facile alternative is ligation of peptide and oligonucleotide fragments using any of a wide range of strategies such as bifunctional cross-linkers, native chemical ligation, click chemistry, hydrazone chemistry and so on. Nonetheless, optimal MALDI matrices for ionization of peptides and oligonucleotides are distinct and the ionization properties of peptide-oligonucleotide conjugates and particularly of phosphorylated conjugates are unpredictable. Toward obviating this confounding factor, we drew on prior studies^{25, 29} that demonstrated that a photocleavable cross-linker could be introduced to provide reliable detection of peptide phosphorylation by MALDI-TOF MS.

Approaches based on exploiting photocleavable crosslinkers have proven to offer a rapid and efficient route for syntheses of many photocleavable bioconjugates for applications in diagnostics and therapeutics.^{44, 56–66} While chemically cleavable bifunctional cross-linkers are readily available, the previously described photocleavable cross-linkers have not been commercialized and require significant synthetic efforts. As a starting point, we chose sulfo-SMCC (sulfosuccinimidyl-4-(N-maleimido-methyl)cyclohexane-1-carboxylate), a commercially available, water-soluble, heterobifunctional cross-linker with amine and sulfhydryl reactivity. A cyclohexane bridge confers added stability to the maleimide group, making sulfo-SMCC an attractive tool for maleimide activation of proteins.⁶⁷ Here, we designed and synthesized a novel sulfo-SMCC-based photocleavable cross-linker (Figure 2). The commonly used commercially available Fmoc-photolinker **1** was deprotected to produce **2**. The amino groups of **2** reacted with the sulfo-NHS functional groups of sulfo-SMCC to form the intermediate photocleavable cross-linker **3**. The carboxyl group of **3** was further activated by treatment with N-hydroxysulfosuccinimide (sulfo-NHS)⁶⁸ under catalysis of N-cyclohexylcarbodiimide, N'-methyl-polystyrene in anhydrous DMF to give target photocleavable cross-linker **4** (**Sulfo-PL-SMCC**) with an overall yield of ~60%. The structure of the key intermediate product **3** was confirmed by ¹H and ¹³C-NMR as well as high-resolution mass spectrometry (HRMS, Figures S2–S4). However, the target product **4** could only be characterized by HRMS due to moisture sensitivity. Good agreement between calculated m/z, 693.1714 for C₂₉H₃₃N₄O₁₄SNa [M-Na]⁺, and measured m/z, 693.1734, were found (Figure S5). Photocleavable cross-linker **4** was then used for the preparation of photocleavable peptide-oligonucleotide conjugates as outlined in Figure 3a. First, 5' amino-modified oligonucleotide **Oligo-NH₂** (5'-NH₂-(CH₂)₆-CTGCGTCGTTTAAGGAAGTAC-3') was reacted with excess **Sulfo-PL-SMCC** in phosphate-buffered saline (PBS) to install a photocleavable maleimide group at the 5'-end. The maleimide-modified oligonucleotide, **Oligo-PL-MAL**, was purified by desalting column and combined with a cysteine-terminated peptide substrate such as CrkL peptide: CGIPEPAHAYAQPQTTTLPAL in PBS with DMF to react with the sulfhydryl group of the terminal cysteine of the peptide, forming conjugate **Oligo-PL-CrkL**. Crude product was dialyzed against water and purified by reverse-phase HPLC to yield photocleavable peptide-oligonucleotide conjugate. HPLC (Figure 3e and 3f) and MALDI-TOF MS (Figure 3b and 3c) analysis each confirmed conversion of **Oligo-NH₂** to intermediate product **Oligo-PL-MAL**. HPLC (Figure 3g and 3h) and MALDI-TOF (Figure 3c and 3d) confirmed formation of peptide-oligonucleotide conjugate **Oligo-PL-CrkL** and purity of the final product. Similarly, three other photocleavable peptide-oligonucleotide conjugates were prepared,

purified and confirmed by MALDI and HPLC (Figure S6). Analytical data obtained with the purified conjugates are summarized in Table 1. The apparent discrepancy between the calculated and observed molecular weights of these conjugates may be attributed to the unavailability of suitable peptide-oligonucleotide standards for MALDI instrument calibration. Chaput et al⁶⁹ also observed a discrepancy when they characterized their peptide-oligonucleotide conjugates by MALDI.

We have previously demonstrated that magnetic beads are a powerful tool for hybrid and solid-phase protein kinase assays, offering separation and suitability to automated procedures as well as favorable analysis by MALDI-TOF MS.^{24–25} To pull out protein kinase products from kinase reaction mixtures by magnetic beads through DNA hybridization for detecting degree of phosphorylation, we investigated the immobilization of the complementary thiol-modified oligonucleotide (5'-HS GTACTTCCTTAAACGACGCAGG-3') on magnetic beads. First, to prevent nonspecific interactions, a poly(ethylene glycol) (PEG) spacer linker was grafted onto the surface of magnetic beads according as previously reported (Figure 4).²⁵ Surface PEG-modified magnetic beads were further reacted with 3-maleimido-propionic acid to produce surface maleimide-functionalized magnetic beads. To immobilize the complementary oligonucleotide onto magnetic beads, the commercially available complementary thiol-modified oligonucleotide was activated by treating with 50 mM TCEP neutral solution for 30 min, and followed by purification with desalting spin columns. The activated thiol-modified oligonucleotide was reacted with surface-maleimide-functionalized magnetic beads in a PBS buffer. The oligonucleotide-coated magnetic beads were further purified by washing with water, and stored in water at 4 °C for the use of hybridization.

To test the feasibility of the DNA hybridization-based protein kinase assay, a solution-phase phosphorylation reaction was performed with the photocleavable peptide-oligonucleotide conjugate, **Oligo-PL-Abl**, and its corresponding kinase c-Abl. After the kinase reaction, the complementary oligonucleotide-coated magnetic beads (**MNP@PEG₈-Oligo^{CS}**) were directly added into the resulting kinase reaction mixture to pull out all phosphorylated and unphosphorylated photocleavable peptide-oligonucleotide conjugates. After magnetic separation, the magnetic beads were further purified by washing with water and subjected to MALDI analysis according as described.²⁵ The recovery efficiency of suspended magnetic beads was estimated to be close to 100% when the magnetic field was applied, based on the rapid accumulation of a tight, brown-colored patch of magnetic beads on the wall of each centrifuge tube, separate from a clear kinase reaction solution.

Figure 5a shows the MALDI spectrum of Abltide released from beads by UV irradiation ($\lambda = 366$ nm) after phosphorylation by c-Abl kinase at 30° C for 1 hour. The expected 80 Da increase in mass relative to the unmodified peptide (Figure 5b, control without the addition of cAbl kinase) was observed. The nearly 100% phosphorylation detected can be ascribed to the kinetic advantages of solution-phase kinase assays. Suggesting that the oligonucleotide tags might not affect the measurement, their influence would have been limited insofar as they were cleaved from the peptides prior to MALDI analysis and we used a matrix that favors the ionization of peptide substrates over oligonucleotides. Under the conditions optimal for peptide analysis, the operating laser voltage was insufficient to ionize the oligonucleotide tags from the bead surfaces. We infer that the presence of oligonucleotide tags likely had little if any effect on detection of the phosphorylated and unphosphorylated ions during the process of MALDI analysis. In a typical kinase reaction mixture, the concentration of c-Abl kinase is 1.75 nM. Preferred fluorescence-based kinase assays such as time resolved-fluorescence resonance energy transfer (TR-FRET) require kinase concentrations of 0.1–20 nM, depending on kinase family, to reach optimized signal to background (S/B) ratios.⁷⁰ Previous studies have shown that the MALDI-TOF MS

technique can yield a thousand-fold S/B ratio, significantly greater than the forty-fold S/B ratio that might be obtained in fluorescence-based kinase assays.^{24, 71} With these considerations in mind, the magnetic bead-mediated solid phase detection offered superior sensitivity and accuracy compared to a conventional solution-phase assay.

Toward implementing a multiplexed kinase assay, we selected three substrate peptide-oligonucleotide conjugates, **Oligo-PL-Abl**, **Oligo-PL-Src** and **Oligo-PL-CrkL**, shown in Table 1. Significantly, Abltide is a high affinity, synthetic substrate selected for phosphorylation by v-Abl kinase,⁷² CrkL peptide is based on the sequence surrounding CrkL Tyr207, a site that is highly phosphorylated by c-Abl and Bcr-Abl in vivo,⁷³ and Src tide, a synthetic substrate selected for affinity for c-Src.⁷² We tested the ATP-competitive, small-molecule, Bcr-Abl and c-Abl tyrosine kinase inhibitor imatinib,⁷⁴⁻⁷⁵ an established single-agent therapeutic for chronic myeloid leukemia and other cancers, for its ability to inhibit phosphorylation of the three peptide substrates by c-Abl kinase. The three purified peptide-oligonucleotide conjugates were combined with c-Abl kinase and 20 μ M ATP and incubated for 1 hour at 30° C in the presence of 0 to 100 μ M imatinib. Then, complementary oligonucleotide-coated magnetic beads were added into each kinase reaction mixture to allow capture of both the unphosphorylated and phosphorylated conjugates via hybridization. The magnetic beads were washed with water, treated with UV to induce photocleavage and released peptides were analyzed by MALDI-TOF MS. Three distinct series of peaks corresponding to Src tide, Abl tide and CrkL peptide were observed (Figure S7). In each series of peaks from reactions in the presence of imatinib, a pair of singly charged peaks displayed 80 Da difference in mass, consistent with each peptide displaying an unphosphorylated form and a phosphorylated form. Percent phosphorylation was calculated based on relative intensity of the phosphorylated and unphosphorylated peptide ions. This phosphorylation ratio may be an underestimate due to suppression of ionization of phosphorylated peptides when MALDI is performed in positive mode. However, our previous work indicates that the IC₅₀ values of imatinib and dasatinib determined by MALDI are comparable to measures made using other methods, such as fluorescence labeling.²⁴ An offsetting contribution may come from the magnetic beads, that may enhance ionization of peptide substrates from the solid surface. In the absence of imatinib, c-Abl kinase phosphorylated ~100% of Abl tide, 72% of Src tide and 30% of CrkL peptide (Figure 6). Here, the signal to background (S/B) ratio was depressed due to measuring amounts of the peptide substrates close to the detection limit of the MALDI instrument used in this study. When the reactions were performed in the presence of imatinib, each peptide displayed a dose-dependent decrease in phosphorylation. When IC₅₀ values were calculated by fitting to the dose-response curves, the three peptide substrates displayed similar values of 0.48 μ M for Abl tide, 0.45 μ M for Src tide and 0.54 μ M for CrkL peptide. The IC₅₀ value of Imatinib against c-Abl is close to the 0.291 μ M that Nishimura et al⁴⁷ determined by a solution-phase kinase assay followed direct MALDI-TOF MS detection using an Abl tide substrate without an oligonucleotide label. This indirectly demonstrates that, despite its larger size, the oligonucleotide tag does not significantly affect the biological activity of the small peptide substrates. Using cell lysates instead of purified kinases provides a more accurate estimate of inhibitor sensitivity and selectivity in a biological setting. Therefore, multiplexed kinase assays with cell lysates are currently under development.

Conclusions

In summary, we have reported the synthesis of a new photocleavable cross-linker and demonstrated its application in the preparation of photocleavable peptide-oligonucleotide conjugates. By capture of photocleavable peptide-oligonucleotide conjugates onto complementary oligonucleotide-coated magnetic beads, photocleavage to release peptides, and MALDI mass spectrometry, we demonstrated the ability to reproducibly measure the

IC₅₀ of Imatinib for c-Abl kinase using 3 different substrates. This DNA-hybridization-mediated multiplexed kinase assay could be directly applied to screening for highly potent and specific inhibitors as an alternative to conventional screening assays. Given sufficiently specific substrate peptides, the approach appears capable of profiling multiple protein kinases in a complex mixture such as cell lysates and thus could enable evaluation of small-molecule kinase inhibitors alone or in combination for their effects on patient samples. Thus, as a route to improving the success of targeting oncogenic tyrosine kinases, we anticipate continued development of these approaches to facilitate drug discovery and validation and to provide a starting point for companion diagnostics.

Experimental section

Materials

Fmoc-photolabile linker (4-[4-(1-(Fmocamino)ethyl)-2-methoxy-5-nitrophenoxy]] butanoic acid, Advanced ChemTech), N-cyclohexyl carbodiimide N'-methyl polystyrene (PS-DCC, loading capability of 2.3 mmole/g, Novabiochem), 3-Maleimido-propionic acid (Bachem), Sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (90%, Sulfo-SMCC) and N-hydroxysulfosuccinimide (90%, Sulfo-NHS) were purchased from Pierce, HCTU coupling reagent (1-[Bis(dimethylamino)methylene]-5-chlorobenzotriazolium-3-oxide hexafluorophosphate, Peptides International), 4-Methylmorpholine (NMM, 99.5+%, Sigma-Aldrich), Piperidine (99%, Sigma-Aldrich), N, N-Dimethylformamide (DMF, anhydrous, 99.8%, Acros Organics), N-Fmoc-amido-dPEG₈-acid (Quanta Biodesign), BcMag Amine-Terminated Magnetic Beads (1 µm, Bioclone Inc.), 1M phosphate buffer (pH 7.2) was prepared by mixing 68.4 mL of 1M Na₂HPO₄ and 31.6 mL of 1M NaH₂PO₄. Recombinant c-Abl (Upstate) was used as received. Imatinib mesylate (Novartis) was provided by Wendy Stock (The University of Chicago). Cysteine-terminated peptide substrates (Abltide: CGGSGGGKGEAIYAAPFAKKKG, Src tide: CAEEIYGEFEAKKKK, CrkL: CGIPEPAHAYAQPQTTPPLPA and Cdc2(6–20): CKVEKIGEGTYGVVYK) were prepared as reported previously.^{21–22} The amino-modified oligonucleotide (5'-NH₂ (CH₂)₆ CCTGCGTCGTTTAAGGAAGTAC-3') and its corresponding complementary thiol-modified oligonucleotide (5'-HS GTACTTCCTTAAACGACGCAGG-3') were purchased from Invitrogen.

HPLC Purification and Analysis

The peptide-oligonucleotide conjugates were purified by C18 reverse phase HPLC with a gradient of 0–40% acetonitrile in 0.1 M TEAA within 30 min. The pure product was collected at the corresponding product peak and volatiles were removed by speedvac. The residue was dissolved in deionized water and used directly for MALDI-TOF MS analysis and re-analysis by HPLC.

MALDI-TOF MS Analysis

Sample preparation for MALDI-TOF analysis was as described,²⁴ except that the dry spotted samples were irradiated by UV light of 366 nm for 40 min prior to addition of α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution onto each sample spot and analysis on a Voyager DE. The phosphorylation ratio of each peptide substrate was determined according to the previously described computational method.²⁴ For MALDI analysis of photocleavable peptide-oligonucleotide conjugates and maleimide-modified oligonucleotide as well as the starting oligonucleotide, matrix solutions were prepared by mixing 3-hydroxypicolinic acid (3-HPA) solution (50 mg/ml in 50:50 water/acetonitrile) and diammonium citrate (50 mg/ml in water) in 9:1 (v/v). Mass spectra were acquired using a 20 kV accelerating voltage, linear operating mode, and positive polarity. The grid voltage was 93% of the accelerating voltage, and the delay time was 350 ns.

Synthesis of Photocleavable Cross-Linkers (Sulfo-PL-SMCC)

Fmoc-protected photolinker **1** (260.0 mg, 0.5 mmol) was dissolved in 20% piperidine in DMF (2.5 mL) and stirred at room temperature for 1 h. The deprotected photolinker was precipitated in a large excess of diethyl ether, isolated by centrifugation and dried under vacuum to give pure deprotected photolinker product, 4-[4-(1-Amino-ethyl)-2-methoxy-5-nitrophenoxy]butyric acid, **2** (129.0 mg, 87%). ¹H-NMR (DMSO-d₆, 500 MHz): δ (ppm) 7.58 (s, Ph), 7.56 (s, Ph), 4.58 (q, CHCH₃), 4.14 (t, CH₂O), 4.02 (s, CH₃O), 2.41 (t, CH₂COOH), 2.03 (m, CH₂(CH₂)₂), 1.40 (d, CH₃CH) (Figure S1).

Deprotected photolinker **2** (10.9 mg, 36.6 μmol) and Sulfo-SMCC (16.8 mg, 38.5 μmol) were dissolved in anhydrous DMF (1000 μL). The reaction mixture was stirred at 37° C for 14.5 h. The reaction solvent was removed by speed vacuum to give dry crude product. The crude product was further washed with water four times to produce less pure target product, which was directly used for the next step, (18.5 mg, 98%). The crude product can be further purified by silica column chromatography with a mixture of CH₂Cl₂-MeOH (10:1) to afford highly pure target product, ([4-(2,5-Dioxo-2,5-dihydro-pyrrol-1-ylmethyl)-cyclohexanecarbonyl]-amino)-ethyl)-2-methoxy-5-nitro-phenoxy]-butyric acid, **3**. ¹H-NMR (DMSO-d₆, 500 MHz): δ (ppm) 12.37 (br, 1H, HOOC), 8.50 (d, 1H, J=7.5 Hz, NHCO), 7.60 (s, 1H, Ph), 7.26 (s, 1H, Ph), 7.12, (s, 2H, C(O)CH=CHCO), 5.42 (m, 1H, CH(CH₃)NH), 4.15 (t, 2H, J= 6.5 Hz, CH₂O), 3.98 (s, 3H, CH₃O), 3.44 (d, 2H, J= 7.5 Hz, CH₂N(CO)₂), 2.48 (t, 2H, J= 7.5 Hz, CH₂COOH), 2.20 (m, 1H, C(O)CH(CH₂)₂), 2.05 (m, 2H, CH₂(CH₂)₂), 1.86-1.68 (m, 4H, COCH(CH₂)₂), 1.61 (m, 1H, CH(CH₂)₃), 1.50 (d, 3H, J=7.0 Hz, CH₃C), 1.38 (m, 1H, CHHCHCH₂N), 1.24 (m, 1H, CHHCHCH₂N), 1.02 (m, 2H, CH₂CHCH₂N). ¹³C NMR (DMSO-d₆, 125.8 MHz): δ (ppm) 175.21 (NHCO), 175.11 (COOH), 172.33 (N(CO)₂), 154.40 (Ar-C-OCH₃), 147.24 (Ar-C-O-CH₂), 141.06 (Ar-C-NO₂), 136.79 (Ar-C-CH-NH), 135.43 (C(O)CH=CH-CO), 110.26 (Ar-C), 109.40 (Ar-C), 69.00 (CH₂O), 57.27 (CH₃O), 44.92 (CH₂N), 44.53 (NHC(O)-CH), 44.10 (CH₃CH), 37.17 (CH₂COOH), 31.15 (CH(CH₂)₃), 30.54, 30.33 (CH₂(CH₂)CH-CH₂), 29.56, 29.06 (CH₂(CH₂)CH-CO), 25.16 (CH₂(CH₂)₂), 22.78 (CH₃CH). HRMS calcd for C₂₅H₃₁N₃O₉. High-resolution mass spectrometry (HRMS) was performed on an Agilent 6410 triple quadrupole mass spectrometer using electrospray ionization (ESI). [M+H]⁺, m/z = 518.2133, found m/z = 518.2153 (ESI); calcd for C₂₅H₃₁N₃O₉Cl [M+Cl]⁻, m/z = 552.1749, found m/z = 552.1809 (ESI).

To a 1.5-mL microcentrifuge tube were added the photocleavable acid **3** (19.0 mg, 36.7 μmol), *N*-hydroxysulfosulfosuccinimide sodium salt (**Sulfo-NHS**, 8.4 mg, 38.7 μmol), *N*-cyclohexylcarbodiimide, *N*'-methyl polystyrene (PS-DCC, 50.0 mg, 65.0 μmol based on the loading capacity of 1.3 mmol/g) and dry DMF (400 μL). The heterogenous reaction mixture was rotated at room temperature for 64 h. The resin was filtered and rinsed with dry DMF twice (200 μL×2). The filtrate was evaporated under vacuum to almost dryness and anhydrous diethyl ether was added to give a powder. The powder product was then washed with anhydrous diethyl ether additional three times and dried under vacuum to give 18.8 mg (71%) of photocleavable cross-linker **4** (**Sulfo-PL-SMCC**) which was used without further purification. HRMS calcd for C₂₉H₃₃N₄O₁₄SN_a [M-Na]⁻, m/z = 693.1714, found m/z = 693.1734.

Synthesis of Photocleavable Peptide-Oligonucleotide Conjugates.⁵²

To activate the oligonucleotide, 300 μL of 100 μM aminomodified oligonucleotide (5'-NH₂-CCTGCGTCGTTTAAGGAAGTAC-3') in water, 33.5 μL of 1M phosphate buffer (pH 7.2) and 120 μL of 40 mM **Sulfo-PL-SMCC** in DMF were mixed together and incubated at 37° C for 1h. The resulting reaction mixture was desalted using NAP-10 columns, yielding photocleavable maleimide-modified oligonucleotide (5'-MAL-PL-

CCTGCGTCGTTTAAGGAAGTAC-3'), which was used in the next step without further purification.

The maleimide-modified oligonucleotide (5'-MAL-PL-CCTGCGTCGTTTAAGGAAGTAC-3', 10 nmol) was incubated with a cysteine-terminated peptide substrate (150 nmol) in 70 μ L of 150 mM PBS buffer (pH 7.2) and DMF mixture (13:1, v/v) under N₂ protection for 6 h. The resulting reaction mixture was evaporated to dryness by Speed Vacuum and further dialyzed against water to remove salt and excess peptide substrate using a Thermo Scientific Slide-a-Lyzer Dialysis Cassette (MWCO: 3.5 KDa) to give crude photocleavable peptide-oligonucleotide conjugate. The crude conjugate was further purified by RP-HPLC to produce pure conjugate. HPLC analysis indicated 90% product conversion. The pure conjugate was further desalted by NAP-10 columns, lyophilized and stored at -20° C.

Preparation of Complementary Oligonucleotide-Coated Magnetic Beads.⁶⁸

BcMAG particles are uniform superparamagnetic beads consisting of silica-coated iron oxide, presenting surface primary amino groups (**MNP@NH₂**). The Superparamagnetic beads were further grafted poly(ethylene glycol) spacers using the PEG linkers, N-Fmoc-amido-dPEG₈-acid according to the previously reported procedure²⁵ to produce PEG surface-modified magnetic beads (**MNP@PEG₈-NH₂**). The PEG-modified magnetic beads were further functionalized by the coupling reaction with 3-Maleimido-propionic acid using the SPPS procedure described elsewhere.²⁵ As a result, maleimide surface modified magnetic beads (**MNP@PEG₈-MAL**) were produced. The maleimide-modified magnetic beads were further reacted with thiol-modified complementary oligonucleotide (5'-SH-GTACTTCCTTAAACGACGAGG-3') to produce complementary oligonucleotide-coated magnetic beads (**MNP@PEG₈-Oligo_{cs}**).

100 μ L of magnetic beads (50 mg/mL, equivalent to 1.25 μ mol NH₂ groups) was placed onto a magnet. The clear solution was removed by a pipette. The beads were washed in turn with water and DMF, and mixed with a mixture of 70 μ L of 400 N-Fmoc-amido-dPEG₈-acid and 1250 μ L of coupling reagent solution containing 0.76 M HCTU, 1.60 M NMM in DMF. The coupling reaction mixture was rotated at room temperature for 40 min, then the beads were separated and washed with DMF three times to remove excess of reactants. The Fmoc protection group was removed by treatment with 20% piperidine in DMF for 30 min. After deprotection, the beads were washed with DMF three times. The surface poly(ethylene glycol) spacer-modified beads (**MNP@PEG₈-NH₂**) was mixed with a mixture of 100 μ L of 1.0 M 3-Maleimido-propionic acid and 1250 μ L of the above-mentioned coupling reagent solution. The coupling reaction was again carried out at room temperature for 40 min. After washing with DMF, surface maleimide-modified magnetic beads with a PEG spacer (**MNP@PEG₈-MAL**) were produced. To immobilize the complementary oligonucleotide, the thiol-modified complementary oligonucleotide was activated according to the following procedure. 10 μ L of 500 μ M thiol-modified oligonucleotide in water was incubated with 1.10 μ L of 500 mM TCEP·HCl (pH 6.9) solution at room temperature for 1 h. After the activation reaction, additional 45 μ L of water was added to the resulting reaction mixture. The diluted activated oligonucleotide mixture was further purified by MicroSpin G-25 columns to produce pure thiol-activated oligonucleotide (5'-HS-GTACTTCCTTAAACGACGAGG-3'). The pure thiol-activated oligonucleotide solution was incubated with the prepared maleimide-modified magnetic beads in 100 μ L of 100 mM PBS buffer (pH 7.2) under N₂ at room temperature for 16 h to produce complementary oligonucleotide-coated magnetic beads (**MNP@PEG₈-Oligo_{cs}**). The oligonucleotide-coated magnetic beads were further washed with water and stored in water at a concentration of 5 mg/mL and kept at 4° C.

***In Vitro* Kinase Assay**

Solution-phase phosphorylation reactions by c-Abl kinase were performed. A typical 50 μ L-kinase reaction contained 50 mM Tris-Cl (pH 7.5), 10 mM $MgCl_2$, 1 mM EGTA, 2 mM DTT, 0.01% Brij-35, 1.2 mg/ml BSA, 0.15 μ M photocleavable peptide-oligonucleotide conjugate, 20 μ M ATP, 0.0272 U recombinant c-Abl, was incubated at 30° C for 1 h. After the kinase reaction mixture was terminated by cooling rapidly down on ice, 5 μ L of magnetic beads were added into the resulting kinase reaction mixture and incubated at room temperature for 75 min. Magnetic beads were separated from the reaction buffer, washed four times with water, and subjected to MALDI-TOF MS analysis.

***In Vitro* Inhibition Assay**

Imatinib was assayed *in vitro* using recombinant c-Abl. Assays with c-Abl was as above with the addition of varying concentrations of Imatinib as well as 75 nM of each photocleavable peptide-oligonucleotide conjugate. A series of kinase reactions with 20 μ M ATP and 0, 1, 10, 100, 500, 10^3 , 10^4 , or 10^5 nM Imatinib were performed for 1 h using c-Abl at 30° C. After the kinase reactions, 5 μ L of magnetic beads were added into each of the resulting kinase reaction mixture and incubated at room temperature for 75 min. The beads were separated and washed with water four times, and stored in 5 μ L of water at 4° C. The degree of phosphorylation for each sample was determined by MALDI-TOF MS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Hunter T. Curr Opin Cell Biol. 2009; 21:140–146. [PubMed: 19269802]
2. Lemmon MA, Schlessinger J. Cell. 2010; 141:1117–1134. [PubMed: 20602996]
3. Grimminger F, Schermuly RT, Ghofrani HA. Nat Rev Drug Discov. 2010; 9:956–970. [PubMed: 21119733]
4. Foran JM. Hematology Am Soc Hematol Educ Program. 2010; 2010:47–55. [PubMed: 21239770]
5. Donato NJ, Peterson LF. Leuk Lymphoma. 2011; 52(Suppl 1):60–80. [PubMed: 21299460]
6. Bixby D, Talpaz M. Leukemia. 2010; 25:7–22. [PubMed: 21102425]
7. Redmond KM, Wilson TR, Johnston PG, Longley DB. Front Biosci. 2008; 13:5138–5154. [PubMed: 18508576]
8. Wu D, Sylvester JE, Parker LL, Zhou GC, Kron SJ. Biopolymers. 2010; 94:475–486. [PubMed: 20593469]
9. Kim M, Shin DS, Kim J, Lee YS. Biopolymers. 2010; 94:753–762. [PubMed: 20564046]
10. Sharma V, Wang Q, Lawrence DS. Biochim Biophys Acta. 2008; 1784:94–99. [PubMed: 17881302]
11. Jia Y, Quinn CM, Kwak S, Talanian RV. Curr Drug Discov Technol. 2008; 5:59–69. [PubMed: 18537568]
12. Pritz S, Meder G, Doering K, Drueckes P, Woelcke J, Mayr LM, Hassiepen U. J Biomol Screen. 2011; 16:65–72. [PubMed: 21148014]
13. Ghadiali JE, Cohen BE, Stevens MM. ACS Nano. 2010; 4:4915–4919. [PubMed: 20731464]
14. Kumar EA, Charvet CD, Lokesh GL, Natarajan A. Anal Biochem. 2011; 411:254–260. [PubMed: 21129358]

15. Mizutani T, Kondo T, Darmanin S, Tsuda M, Tanaka S, Tobiume M, Asaka M, Ohba Y. Clin Cancer Res. 2010; 16:3964–3975. [PubMed: 20670950]
16. Devkota AK, Kaoud TS, Warthaka M, Dalby KN. Curr Protoc Mol Biol. 2010; Chapter 18(Unit 18):17. [PubMed: 20583097]
17. Zaman GJ, Garritsen A, de Boer T, van Boeckel CA. Comb Chem High Throughput Screen. 2003; 6:313–320. [PubMed: 12769674]
18. Li M, Luraghi P, Amour A, Qian XD, Carter PS, Clark CJ, Deakin A, Denyer J, Hobbs CI, Surby M, Patel VK, Schaefer EM. Anal Biochem. 2009; 384:56–67. [PubMed: 18762159]
19. Yu YH, Anjum R, Kubota K, Rush J, Villen J, Gygi SP. Proc Natl Acad Sci U S A. 2009; 106:11606–11611. [PubMed: 19564600]
20. Houseman BT, Huh JH, Kron SJ, Mrksich M. Nat Biotechnol. 2002; 20:270–274. [PubMed: 11875428]
21. Sylvester JE, Kron SJ. Mol Cancer Ther. 2010; 9:1469–1481. [PubMed: 20423990]
22. Wu D, Mand MR, Veach DR, Parker LL, Clarkson B, Kron SJ. Anal Biochem. 2008; 375:18–26. [PubMed: 18194660]
23. Wu D, Nair-Gill E, Sher DA, Parker LL, Campbell JM, Siddiqui M, Stock W, Kron SJ. Anal Biochem. 2005; 347:67–76. [PubMed: 16236241]
24. Zhou GC, Sylvester JE, Wu D, Veach DR, Kron SJ. Anal Biochem. 2011; 408:5–11. [PubMed: 20807497]
25. Zhou G, Yan X, Wu D, Kron SJ. Bioconjugate Chem. 2010; 21:1917–1924.
26. Zhu H, Bilgin M, Bangham R, Hall D, Casamayor A, Bertone P, Lan N, Jansen R, Bidlingmaier S, Houfek T, Mitchell T, Miller P, Dean RA, Gerstein M, Snyder M. Science. 2001; 293:2101–2105. [PubMed: 11474067]
27. Greis KD. Mass Spectrom Rev. 2007; 26:324–339. [PubMed: 17405133]
28. Akita S, Umezawa N, Kato N, Higuchi T. Bioorg Med Chem. 2008; 16:7788–7794. [PubMed: 18656369]
29. Parker LL, Brueggemeier SB, Rhee WJ, Wu D, Kent SBH, Kron SJ, Palecek SP. Analyst. 2006; 131:1097–1104. [PubMed: 17003856]
30. Kim YP, Oh E, Oh YH, Moon DW, Lee TG, Kim HS. Angew Chem-Int Edit. 2007; 46:6816–6819.
31. Shults MD, Kozlov IA, Nelson N, Kermani BG, Melnyk PC, Shevchenko V, Srinivasan A, Musmacker J, Hachmann JP, Barker DL, Lebl M, Zhao CF. ChemBioChem. 2007; 8:933–942. [PubMed: 17471478]
32. Kubota K, Anjum R, Yu YH, Kunz RC, Andersen JN, Kraus M, Keilhack H, Nagashima K, Krauss S, Paweletz C, Hendrickson RC, Feldman AS, Wu CL, Rush J, Villen J, Gygi SP. Nat Biotechnol. 2009; 27:933–940. [PubMed: 19801977]
33. Dierck K, Machida K, Voigt A, Thimm J, Horstmann M, Fiedler W, Mayer BJ, Nollau P. Nat Methods. 2006; 3:737–744. [PubMed: 16929320]
34. Sahoo H, Hennig A, Florea M, Roth D, Enderle T, Nau WM. J Am Chem Soc. 2007; 129:15927–15934. [PubMed: 18044894]
35. Zondlo SC, Gao F, Zondlo NJ. J Am Chem Soc. 2010; 132:5619–5621. [PubMed: 20361796]
36. Lukovic E, Gonzalez-Vera JA, Imperiali B. J Am Chem Soc. 2008; 130:12821–12827. [PubMed: 18759402]
37. Wang Q, Zimmerman EI, Toutchkine A, Martin TD, Graves LM, Lawrence DS. ACS Chem Biol. 2010; 5:887–895. [PubMed: 20583816]
38. Phanstiel DH, Brumbaugh J, Wenger CD, Tian S, Probasco MD, Bailey DJ, Swaney DL, Tervo MA, Bolin JM, Ruotti V, Stewart R, Thomson JA, Coon JJ. Nat Methods. 2011; 8:821–827. [PubMed: 21983960]
39. Shults MD, Janes KA, Lauffenburger DA, Imperiali B. Nat Methods. 2005; 2:277–283. [PubMed: 15782220]
40. McLachlin DT, Chait BT. Curr Opin Chem Biol. 2001; 5:591–602. [PubMed: 11578935]
41. Woo EM, Fenyo D, Kwok BH, Funabiki H, Chait BT. Anal Chem. 2008; 80:2419–2425. [PubMed: 18321079]

42. Li X, Foley EA, Molloy KR, Li Y, Chait BT, Kapoor TM. *J Am Chem Soc.* 2012; 134:1982–1985. [PubMed: 22239320]
43. Parker L, Engel-Hall A, Drew K, Steinhardt G, Helseth DL, Jabon D, McMurry T, Angulo DS, Kron SJ. *J Mass Spectrom.* 2008; 43:518–527. [PubMed: 18064576]
44. Parker LL, Kurutz JW, Kent SBH, Kron SJ. *Angew Chem-Int Edit.* 2006; 45:6322–6325.
45. Parker LL, Schilling AB, Kron SJ, Kent SBH. *J Proteome Res.* 2005; 4:1863–1866. [PubMed: 16212443]
46. Kim EM, Kim J, Kim YG, Lee P, Shin DS, Kim M, Hahn JS, Lee YS, Kim BG. *J Pept Sci.* 2011; 17:392–397. [PubMed: 21480437]
47. Kondo N, Nishimura S. *Chem Eur J.* 2009; 15:1413–1421. [PubMed: 19115309]
48. Kinumi T, Niki E, Shigeri Y, Matsumoto H. *J Biochem.* 2005; 138:791–796. [PubMed: 16428308]
49. Tung CH, Stein S. *Bioconjugate Chem.* 2000; 11:605–618.
50. Lonnberg H. *Bioconjugate Chem.* 2009; 20:1065–1094.
51. Lu K, Duan QP, Ma L, Zhao DX. *Bioconjugate Chem.* 2010; 21:187–202.
52. Lapiene V, Kukolka F, Kiko K, Arndt A, Niemeyer CM. *Bioconjugate Chem.* 2010; 21:921–927.
53. Niemeyer CM. *Angew Chem-Int Edit.* 2010; 49:1200–1216.
54. Fraley AW, Pons B, Dalkara D, Nullans G, Behr JP, Zuber G. *J Am Chem Soc.* 2006; 128:10763–10771. [PubMed: 16910671]
55. Schweller RM, Constantinou PE, Frankel NW, Narayan P, Diehl MR. *Bioconjugate Chem.* 2008; 19:2304–2307.
56. Tang XJ, Maegawa S, Weinberg ES, Dmochowski IJ. *J Am Chem Soc.* 2007; 129:11000–11001. [PubMed: 17711280]
57. Olejnik J, Ludemann HC, Krzymanska-Olejnik E, Berkenkamp S, Hillenkamp F, Rothschild KJ. *Nucleic Acids Res.* 1999; 27:4626–4631. [PubMed: 10556319]
58. Tang XJ, Dmochowski IJ. *Nat Protoc.* 2006; 1:3041–3048. [PubMed: 17406566]
59. Tang XJ, Dmochowski IJ. *Angew Chem-Int Edit.* 2006; 45:3523–3526.
60. Saran D, Burke DH. *Bioconjugate Chem.* 2007; 18:275–279.
61. Yan FN, Chen LH, Tang QL, Rong W. *Bioconjugate Chem.* 2004; 15:1030–1036.
62. Bai XP, Kim SB, Li ZM, Turro NJ, Ju JY. *Nucleic Acids Res.* 2004; 32:535–541. [PubMed: 14744978]
63. Ruparel H, Bi LR, Li ZM, Bai XP, Kim DH, Turro NJ, Ju JY. *Proc Natl Acad Sci U S A.* 2005; 102:5932–5937. [PubMed: 15829589]
64. Bai XP, Li ZM, Jockusch S, Turro NJ, Ju JY. *Proc Natl Acad Sci U S A.* 2003; 100:409–413. [PubMed: 12515854]
65. Olejnik J, Sonar S, Krzymanska-Olejnik E, Rothschild KJ. *Proc Natl Acad Sci U S A.* 1995; 92:7590–7594. [PubMed: 7638235]
66. Ouyang XH, Shestopalov IA, Sinha S, Zheng GH, Pitt CLW, Li WH, Olson AJ, Chen JK. *J Am Chem Soc.* 2009; 131:13255–13269. [PubMed: 19708646]
67. Christie RJ, Anderson DJ, Grainger DW. *Bioconjugate Chem.* 2010; 21:1779–1787.
68. Trester-Zedlitz M, Kamada K, Burley SK, Fenyo D, Chait BT, Muir TW. *J Am Chem Soc.* 2003; 125:2416–2425. [PubMed: 12603129]
69. Williams BAR, Chaput JC. *Curr Protoc Nucleic Acid Chem.* 2010; Chapter 4(Unit4.41)
70. Blouin J, Roby P, Arcand M, Beaudet L, Lipari F. *Curr Chem Genomics.* 2011; 5:115–121. [PubMed: 21966344]
71. Appelblom H, Nurmi J, Soukka T, Pasternack M, Penttila KE, Lovgren T, Niemela P. *J Biomol Screen.* 2007; 12:842–848. [PubMed: 17579123]
72. Zhou S, Carraway KL, Eck MJ, Harrison SC, Feldman RA, Mohammadi M, Schlessinger J, Hubbard SR, Smith DP, Eng C, Lorenzo MJ, Ponder BAJ, Mayer BJ, Cantley LC. *Nature.* 1995; 373:536–539. [PubMed: 7845468]
73. de Jong R, ten Hoeve J, Heisterkamp N, Groffen J. *Oncogene.* 1997; 14:507–513. [PubMed: 9053848]

74. Buchdunger E, Zimmermann J, Mett H, Meyer T, Muller M, Druker BJ, Lydon NB. Cancer Res. 1996; 56:100–104. [PubMed: 8548747]
75. Deininger M, Buchdunger E, Druker BJ. Blood. 2005; 105:2640–2653. [PubMed: 15618470]

Figure 1.

Schematic representation of a DNA hybridization-mediated multiplexed protein kinase assay. Different photocleavable peptide-oligonucleotide conjugates are pooled and incubated with their corresponding kinases. After kinase reactions, the complementary oligonucleotide-coated magnetic beads are directly added into the resulting kinase reaction mixture to pull out all phosphorylated and unphosphorylated photocleavable peptide-oligonucleotide conjugates through hybridization. The hybridized oligonucleotide-peptide conjugated magnetic beads are subjected to photocleavage by UV light. Both phosphorylated and unphosphorylated substrate peptides can be detected by MALDI with a characteristic 80 Dalton difference in mass between each set of two peaks.

Figure 2.

Synthesis of the photocleavable heterobifunctional cross-linker (**Sulfo-PL-SMCC**): a) 20% piperidine in DMF, 1h, 87%; b) dry DMF, 37 °C, 14.5 h, 98%; c) dry DMF, 64 h, 71%; DMF = N, N-dimethylformamide.

Figure 3.

(a) Synthetic route for the photocleavable peptide-oligonucleotide conjugates including two-step reactions: the insertion of a photocleavable maleimide group at the 5' end of oligonucleotide via amine-Sulfo-NHS ester reaction and coupling reaction between maleimide-modified oligonucleotide and peptide substrate via Michael addition. The MALDI spectra, (b)–(d), and HPLC chromatographs, (e)–(g), of the representative purified peptide-oligonucleotide conjugate, **Oligo-PL-CrkL**, 3'-CATGAAGGAATTTGCGTCC-PL-CGIPEPAHAYAQPQTTPPLPA, desalting column-purified maleimide-modified oligonucleotide, **Oligo-PL-MAL**, and the starting amino-modified oligonucleotide precursor, **Oligo-NH₂**. The HPLC chromatograph (h) of the representative crude peptide-oligonucleotide conjugate, **Oligo-PL-CrkL**, was inserted for comparison.

Figure 4.
 Synthetic route for the complementary oligonucleotide-coated magnetic beads with a PEG spacer.

Figure 5. MALDI spectra of peptide fragments generated by photocleavage from photocleavable peptide-oligonucleotide conjugates that were pulled out by the corresponding complementary oligonucleotide-coated magnetic beads after kinase reactions in the presence (a) or absence (b) (provided as control) of c-Abl kinase. Predicted photocleavage site and theoretical molecular mass of the corresponding generated peptide fragments were also given for each experiment.

Figure 6.

Inhibition assays using Imatinib against c-Abl kinase using the three different photocleavable substrate peptide-oligonucleotide conjugates and MALDI-TOF MS analysis. The degree of phosphorylation was calculated from the ratio of phosphorylated and unphosphorylated peak intensities for each peptide substrate. Each data point is the average of three spectra. ATP concentration was 20 μ M. Error bars show s. d. A Sulfo-SMCC-based photocleavable cross-linker was synthesized and further used to prepare photocleavable peptide-oligonucleotide bioconjugates, which can re-release peptides under UV irradiation and facilitate detection by MALDI-TOF MS with high sensitivity and specificity.

Table 1

Molecular mass and HPLC elution time of photocleavable peptide-oligonucleotide conjugates and their corresponding maleimide-modified and original oligonucleotides as well as the peptide sequences used for preparation of the conjugates.

Sample code	Sequence of peptide terminated by oligonucleotide	Molecular mass (Da)		HPLC elution time (min)
		Calculated	MALDI	
Oligo-PL-Abl	CGSGGGKGEIYAAPFAKKKG	9482.8	9502.68	27.04
Oligo-PL-Src	CAEEIYGEFEAKKKK	9330.8	9352.37	25.57
Oligo-PL-CrkL	CGIPEPAHAYAQPQTTPPLPA	9592.8	9598.78	27.65
Oligo-PL-Cdc2	CKVEKIGEGTYGVVYK	9202.9	9224.95	27.33
Oligo-NH2		6930.6	6938.91	18.21
Oligo-PL-MAL		7429.8	7475.92	28.55